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# Request for grant of a patent

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Cardiff Road
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NP10 8QQ

1. Your reference — P32707-/CHO/BPU

2. Patent application number (The Patent Office will fill in this part) — 0228465.1 — 6 DEC 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames) —
The Queens University of Belfast
University Road
Belfast
BT7 1NN

Patents ADP number (if you know it) — 5578786005

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — A Method of Treating Disease

5. Name of your agent (if you have one) — Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode) —
Scotland House
165-169 Scotland Street
Glasgow
G5 8PL

Patents ADP number (if you know it) — 1198015

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

| Country | Priority application number (if you know it) | Date of filing (day / month / year) |
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| | | |

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing (day / month / year) |
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| | |

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:
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b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
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| Priority documents | |
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| Request for preliminary examination and search (Patents Form 9/77) | |
| Request for substantive examination (Patents Form 10/77) | |
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11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company

Date 6 December 2002

12. Name and daytime telephone number of person to contact in the United Kingdom: Barry Purdy 0141 307 8400

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# A Method of Treating Disease

The invention relates to a method of treating disease. In particular, the invention relates to the treatment of inflammatory and other diseases of the type having a pathogenesis which involves endogenous production of any of cytokines IL-12, IL 23 or IL 27.

Cytokines are a unique family of growth factors. Secreted primarily from leukocytes, cytokines stimulate both the humoral and cellular immune responses, as well as the activation of phagocytic cells. Cytokines secreted from lymphocytes are termed lymphokines, whereas those secreted by monocytes or macrophages are termed monokines. Many of the lymphokines are also known as interleukins (IL's), since they are not only secreted by leukocytes, but are also able to affect the cellular responses of leukocytes. Specifically, interleukins are growth factors targeted to cells of hematopoietic origin. One of the interleukins, IL-12, is a pro-inflammatory cytokine interleukin. This cytokine is predominantly secreted either as a αβ heterodimeric form or as a ββ homodimeric form. Both dimer forms bind the IL-12-receptor on target cells but differ in the spectrum of biological activities induced. The αβ form is crucial for generation of cell-mediated immunity against parasites, viruses and bacteria, but contributes also to destructive effects in pathogenesis of autoimmune diseases,e.g. MS, RA and inflammatory bowel disease. The ββ form

has been shown to be instrumental in virus-induced inflammation, and in excessive epithelial airway inflammation seen in asthma. Thus, both forms of IL-12 are disease-promoting factors in a variety of conditions. Recently, two novel cytokines have been discovered, named interleukin-23 and interleukin-27 that apparently belong to the IL-12 subclass of cytokines based on structural relationships. Both IL-23 and IL-27 share with IL-12 a typical heterodimeric structure and are invloved in a similar array of immune responses.

Celebrex is a diaryl-substituted pyrazole. It is a nonsteroidal anti-inflammatory drug (NSAID) that is indicated for the treatment of osteoarthritis, rheumatoid arthritis, for the management of acute pain in adults for the treatment of primary dysmenorrhea. The mechanism of action of CELEBREX is believed to be due to inhibition of prostaglandin synthesis, primarily via inhibition of cyclooxgenase-2 (COX-2). Scientific literature indicates that CELEBREX displays antitumor effects by sensitizing cancer cells to apoptosis. A recent paper has indicated that CELEBREX blocks the endoplasmic reticulum (ER) $Ca^{2+}$-ATPases, and it has been suggested that this $Ca^{2+}$ perturbation may be part of the signaling mechanism by which CELEBREX triggers apoptosis. This $Ca^{2+}$ perturbation effect seems to be unique to CELEBREX and was not seen with any of the other COX inhibitors (e.g. aspirin, ibuprofen, naproxen etc.)

In a first aspect, the invention provides a method of treating disease having a pathogenesis which includes endogenous production of any of cytokines IL-12, IL 23 or IL-27, the method comprising a step of treating an individual with an endoplasmic reticulum (ER) $Ca^{2+}$ perturbation reagent.

In a second aspect, the invention provides the use of an ER $Ca^{2+}$ perturbation reagent in the manufacture of a medicament for the treatment of disease having a pathogenesis which includes endogenous production of any of cytokines IL-12, IL-23 or IL-27.

In a third aspect, the invention provides the use of an ER $Ca^{2+}$ perturbation reagent for the treatment of disease having a pathogenesis which includes endogenous production of any of cytokines IL-12, IL-23 or IL-27.

In a forth aspect, the invention relates to a method of inhibiting the formation of one or more cytokines in an individual, which method comprises the step of treating an individual with ER $Ca^{2+}$ perturbation reagent. In one embodiment, the cytokines are selected from IL-12, IL-23 and IL-27.

In a fifth aspect, the invention relates to the use of an ER $Ca^{2+}$ perturbation reagent to inhibit the formation of one or more cytokines in an individual. In one embodiment the cytokines are selected from IL-12, IL-23 and IL-27.

In a preferred embodiment, the disease is an inflammatory disease. More preferably, the disease is a disease in which one or more endogenously produced IL-12 forms play a disease promoting role. Typically, the IL-12 forms are αβ heterodimeric and ββ homodimeric forms.

In one embodiment, diseases in which cyclooxygenase-2 (COX-2) is reported to play a substantial disease promoting role are disclaimed.

In one embodiment, the inflammatory disease is a disease in which the endogenous production of one or both of αβ and ββ forms of IL-12 is known to lead to disease in a COX-2 independent manner.

The invention also relates to a method of inhibiting the production of one or more cytokines in an individual in a post-translational manner, which method comprises a step of treating an individual with ER $Ca^{2+}$ perturbation reagent.

Preferably, the disease is selected from the group consisting of infectious diseases; bacterial protozoal or virus-induced inflammation; epithelial airway inflammation such as asthma; allergic disease; autoimmune disease such as MS, RA and Inflammatory Bowel Disease; and -all conditions in which endogenously produced IL-12 α/β or ββ forms are thought to play a disease-promoting role, including:

Pulmonary fibrosis
Pulmonary tuberculosis
Asthma
Sarcoidosis
Leprosy
Schistosomiasis
Lupus erythematosis
Lupus nephritis
Allograft rejection
Airway inflammation
Respiratory syncytial virus infection
Multiple sclerosis
Alzheimer's disease
Abortion (women with recurrent pregnancy loss)
Certain vaccines aimed at inducing TH2-type immune responses
Experimental autoimmune myocarditis
Tuberculosis
Psoriatic arthritis
Rheumatoid arthritis
Osteoarthritis
Colonic inflammation (colitis)
Chron's Disease
Inflammatory bowel disease
Atopic dermatitis, AD (chronic stage)
Inflammatory skin disease
Insulin dependent diabetes mellitus Type I and II
Endotoxaemia
Exposure to organic dust
Periodontal diseases
Nephrotic syndrome
Hepatocellular damage in chronic hepatitis C

Primary biliary cirrhosis

Cancer patients (Various cancers, and various stages in cancer that are typically accompanied with dysregulated IL-12, IL-23 and/or or IL-27 production)

ANCA associated vasculitis and sepsis

Experimental crescentic glomerulonephritis

Atherosclerosis

Acute viral myocarditis

Autoimmune myocarditis

Experimental autoimmune myastenia gravis

Uveitis (as Behret's disease)

Thyroiditis and Grave's disease

Thyroid autoimmune disease

Myelopathy (HTLV-I-associated myelopathy)

Symptomatic transient hypogammaglobulinaemia of infancy (THI)

Selective IgA deficiency (SIgAD)

Schizophrenia

Primary malignant melanoma

Abdominal aortic aneurysm

Autoimmune thrombocytopenic purpura

Heatstroke

Meningococcal sepsis

Septic shock

Meningoencephalitis

Bacterial meningitis

Pregnancy

Pre-eclampsia

HELLP syndrome (hemolysis, elevated liver function test and low platelet counts

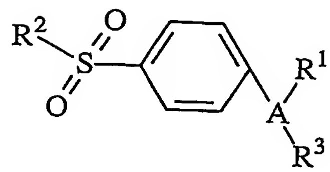
Endometriosis

Acute pancreatitis
Lung fibrosis induced by silica particles
Scleroderma
Sjogren's syndrome
Ankylosis spondylitis
Hashimoto's thyroiditis
Autimmune anemias
Goodpasture's syndrome
Addinson's disease
Autoimmune hemolitic anemia
Spontaneous infertility (sperm)
Poststreptococcal glomerulonephritis
Autoimmune neuritis (Guillian-Barrd syndrome)
Sialadenitis
Brucellosis
Chickenpox and related viral diseases
Helicobacter Pyloris-induced gastritis
Common Variable Immunodeficiency (CVI)

In one embodiment, the disease is a conditions characterized by dysregulation of IL-12, IL-23 or IL-27 production conferred by polymorphisms in their respective genes, or by polymorphisms in genes involved in the biological activation or signal transduction pathway of these cytokines.

In one embodiment, the ER $Ca^{2+}$ perturbation reagent is selected from the compounds of Formula I:

Formula I



wherein A is a substituent selected from partially unsaturated or unsaturated hetrocyclyl and partially unsaturated or unsaturated carbocyclic rings; wherein $R^1$ is at least one substituent selected from hetercyclyl, cycloalkyl, cycloalkenyl and aryl, wherein $R^1$ is optionally substituted at a substitutable position with one or more radicals selected from alkyl, haloalkyl, cyano, carboxyl, alkoxycarbonyl, hydroxyl, hydroxyalkyl, amino, alkylamino, arylamino, nitro, alkoxyalkyl, alkylsulfinyl, halo, alkoxy and alkylthio; wherein $R^2$ is methyl or amino; and wherein $R^3$ is a radical selected from hydrido, halo, alkyl, alkenyl, oxo, cyano, carboxyl, cyanoalkyl, heterocyclyloxy, alkyloxy, alkylthio, alkylcarbonyl, cycloalkyl, aryl, haloalkyl, heterocyclyl, cycloalkenyl, aralkyl, hetrocyclylalkyl, acyl, alkythioalkyl, hydroxyalkyl, alkoxycarbonyl, arylcarbonyl, aralkylcarbonyl, aralkenyl, alkoxyalkyl, arylthioalky, aryloxyalkyl, aralkylthioalky, aralkoxyalkyl, alkoxyaralkoxyalkyl, alkoxycarbonalkyl, aminocarbonyl, aminocarbonylalkyl, alkyaminocarbonyl, N-arylaminocarbonyl, N-alkyl-N-arylaminocarbonyl, alkylaminocarbonylalkyl, carboxyalkyl, alkylamino, N-arylamino, N-aralkylamino, N-alkyl-N-aralkylamino, N-alkyl-N-arylamino, aminoalkly, alkylaminoalkyl, N-

arylaminoalkyl, N-aralkylaminoalkyl, N-alkyl-N-aralkylaminoalky, N-alkyl-N-arylaminoalkyl, aryloxy, aralkoxy, arylthio, aralkylthio, alkylsulfinyl, alkylsulfonyl, aminosulfonyl, alkylaminosulfonyl, N-arylaminosulfonyl, arylsulfonyl, N-alkyl-N-arylaminosulfonyl; or a pharmaceutically-acceptable salt thereof.

In a preferred embodiment, the ER $Ca^{2+}$ perturbation reagent is selected from the compounds and compositions described in US Patent 5,972,986, Column 3, line 34 to Column 10, line 32. In a particularly preferred embodiment, the ER $Ca^{2+}$ perturbation reagent is a diaryl- substituted pyrazole marketed under the brand name CELEBREX (Celecoxib). CELEBREX is chemically designated as 4-[5-(4-methylpheny)-3-(trifluoromethyl)-IH-pyrazol-I-yl] benzenesulfonamide.

Alternatively, the ER $Ca^{2+}$ perturbation reagent may be thapsigargin or A23187.

The invention will be more clearly understood from the following description of some embodiments thereof, given by way of example only:

Recombinant cell lines that secrete various forms of IL-12 under control of tightly regulated promoters were generated. It was observed that treatment of these cell lines with an ER $Ca^{2+}$ perturbation reagent such as thapsigarin inhibited secretion of both the $\alpha\beta$ and $\beta\beta$ forms of IL-12.Given the $Ca^{2+}$ perturbation

activity of CELEBREX, this compound was also tested on assembly of IL-12, and found that it exerts a similar inhibitory effect on the secretion of the αβ and ββ forms of IL-12. There is a total block in the secretory production of both dimer forms of IL-12, and maximal effects are obtained with the normal physiological working concentration of CELEBREX in the absence of any apparent toxic effects as measured with the MTT assay. These affects are conferred in a post-transcriptional and post-translation manner as there is no effect on mRNA of IL-12. Though the precise mechanism by which CELEBREX exerts these effects is still under investigation, and without being bound by theory, evidence has been produced to support a $Ca^{2+}$-dependent disturbance in the folding pathway of IL-12 due to impaired activity of certain chaperones in the ER.

The inhibitory effect of CELEBREX on formation of the αβ and ββ forms of IL-12 in vitro indicates that this drug is of interest for the treatment of inflammatory conditions in which endogenous production of these IL-12 forms is known to lead to disease in a COX2-independent manner, including MS, IBD, virus-induced inflammation and asthma.

IL-12 is a member of a family of cytokines that includes two recently discovered members IL-23 and IL-27. All of these cytokines have a typical heterodimeric structure and display an array of both overlapping and distinct activities. It is thought

that also IL-23 and IL-27 may contribute to destructive inflammation in various conditions. Given the similar subunit assembly configuration of these 3 cytokines, it is likely that CELEBREX will exert similar inhibitory effects on assembly of IL-23 and IL-27. If confirmed, this would imply that a single drug could be used for the simultanious inhibition of 3 cytokines. Most anti-cytokine drugs work by inhibiting transcription of mRNA. To our knowledge this is the first demonstration of a drug that inhibits cytokine formation in a post-translational manner on the level of folding and secretion of the protein, i.e. by perturbation.

Experimental methods

Materials. Celecoxib (Celebrex) was obtained from Hefei Sceneri Chemical Co.; thapsigargin was obtained from Calbiochem and A23187 from Sigma.

Cell culture. HEK293 IL-12 β/β and α/β producing cell lines were maintained in a $CO_2$ incubator at 37 °C (5% $CO_2$). Cells were cultured in DMEM medium supplemented with 10% foetal bovine serum.

Transfection and production of stable cell lines. Human embryonic kidney cells previously transfected with a pVgRXR construct that encodes a functional human retinoid X/ecdysone receptor, were obtained from Invitrogen (EcR-293). Cassettes coding for the human IL-12 α and β-chains were amplified from the respective full-length cDNAs and fused with a C-

terminal hexahistidine-tag ($H_6$). These were introduced in expression vectors and transfected with FuGENE (Boehringer Mannhein) into EcR-293 cells. Clones were selected with the antibiotics Zeocin (400μg/ml) and G418 (600μg/ml). Protein expression was induced with 5μM Ponasterone A (Ecdysone analog).

Capture of α/β- and β/β-H6-chaperone complexes on $Ni^{2+}$-NTA.

Following induction with Ponasterone A, cells were lysed. - α/β and β/β -$H_6$-chaperone complexes were captured on $Ni^{2+}$-NTA agarose. The gel was washed 5 times with buffer A (100mM NaH2PO4, 10mM TrisHCl, 8M urea, pH 6.3), and elution was carried out with buffer B (same as Buffer A, but pH 4.3). Complexes were boiled in SDS loading solution + DTT. Proteins were separated by 4-15% SDS-PAGE and transferred to PVDF membranes. Detection was carried out using anti-p35 antibody G161-566.14 (Pharmingen). Membranes were stripped and re-probed successively with anti-chaperone antibodies (α -CRT, α -Grp78, α -Grp94 & α -CNX; StressGen).

Treatment with inhibitors.

Cells were cultured in 12 well plates with a density of $10^5$ cells per well. Cells were treated with reagents at different concentrations for two hours and afterwards the cells were induced with 5 μM of Ponasterone A to produce the β/β and α/β chains. Inhibitors were added to the culture medium of induced cells at the following concentrations:

between 10 μM and 100 μM for celecoxib, between 0.1 to 30 μM for A23187 and between 0.5 to 45 μM for thapsigargin. Medium was collected and lysates prepared after 16 hours of induction.

Western Blot and immunodetection of IL-12 β/β and α/β.

Culture medium, lysates and immunoprecipitated fractions were mixed with non-reducing or reducing loading solution and were subjected to 4-15% SDS-PAGE. Proteins were transferred from gels to PVDF membranes by electroblot. Membranes were blocked in 2% casein in TBS buffer. Immuno-detection was performed with the mouse monoclonal anti α-chain antibody purchased from PharMingen (G161-566) and the mouse monoclonal anti β-chain antibody purchased from Abcam (1-2A1). The secondary antibody was goat anti-mouse IgG HRP-conjugated obtained from Jackson Immunoresearch. The detection of IL-12 forms was carried out using the ECL-plus kit from Amersham-Pharmacia.

Cytotoxicity test.

The MTT (3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl tetrazolium bromide) reagent was use to determinate the cytotoxicity level after treatment of cells with the inhibitors. The test was performed in 96 well plates to which different concentrations of inhibitors were added. After two hours the cells were induced with Ponasterone A. The MTT reagent was added to the cells after 16 hours of induction and the absorbance measurement was carried out in a spectrophotometer at 570 nm.

## Experimental findings

IL-12 is a secretory protein. Secretory proteins are defined as proteins that are released by cells into the extracellular milieu, and that exert their biological activity by binding onto a specific membrane receptor of target cells. 'Folding' (i.e. generation of a correct three-dimensional structure) of secretory proteins, such as IL-12, typically occurs in a membrane-surrounded cell organelle, named the endoplasmic reticulum (ER). The ER is specifically enriched in chaperones, thioredoxin-type isomerases and proteins involved in glycosylation pathways. An important role of these factors is to assist in ensuring correct folding of secretory proteins during their transit in the ER prior to their secretion into the extracellular milieu. Improperly folded secretory proteins are generally retained in the ER and subsequently degraded by proteases and components of the cytosolic proteasome. It was hypothesised that the use of selected pharmacological agents that interfere with the proper functioning of 'folding'-assisting factors in the ER could be used to inhibit proper folding, and, hence, secretion of IL-12.

As a first step, different tightly controlled ecdysone-inducible recombinant cell lines expressing functional C-terminally hexahistidine-tagged IL-12 $\alpha/\beta$ (heterodimer) and IL-12 $\beta/\beta$ (homodimer) chains were developed. The use of such recombinant cell lines alleviates some of the problems related to the

use of natural producer cells of IL-12 (e.g. restricted availability, lack of reproducibility etc). These recombinant cell lines were used as a means to study the processes that determine regulation of folding, assembly and secretion of IL-12 homo- and heterodimers. The following inhibitors were used: (i) thapsigargin (an ER $Ca^{2+}$-ATPase inhibitor), and (ii) the ionophore A23187 and (iii) celecoxib (a putative ER $Ca^{2+}$ perturbating reagent), each over a wide range of concentrations.

Following a 16-hr treatment of cells with these inhibitors, culture medium was collected and the presence of secreted IL-12 forms was detected by means of non-reducing SDS-PAGE and western immunoblot. It was found that neither the α/β nor the β/β dimer forms of IL-12 were present in the culture medium of cells treated with thapsigargin when this was added over a concentration range of 0.1 μM to 15 μM. The amount of extracellularly secreted IL-12 dimer forms produced by thapsigargin-treated cells was <5% of that produced by untreated cells (maximal suppression was observed for all concentrations of thapsigargin greater than or equal to 0.1 μM). Similarly, the calcium ionophore A23187 suppressed formaton of secreted IL-12 dimer forms when it was used over a concentration range of 0.1 μM to 30 μM, with maximal suppression (>95% compared to untreated cells) from 1 μM. Toxicity conferred by these inhibitors over the test period of 16 hr as measured with the MTT test was observed for concentrations of thapsigargin >5-10 μM and for concentrations of A23187 >10 μM. Thus, the maximal

suppression of secreted IL-12 dimer production is achieved at an inhibitor concentration at which toxic effects are totally absent, showing that both IL-12-suppressive and cell-toxic effects conferred by these inhibitors are independent. Secretion of IL-12 α and β monomer forms was suppressed by neither thapsigargin nor A23187.

Both thapsigargin and A23187 are likely to exert these effects by decreasing the concentration of $Ca^{2+}$ in the ER. It is likely that the resulting suboptimal concentration of $Ca^{2+}$ in the ER blocks the activity of $Ca^{2+}$-dependent chaperones and folding-assisting proteins involved in the dimer formation of IL-12. Since Celecoxib is thought to disturb the $Ca^{2+}$ concentration in the ER in much a similar way to the mode of action of thapsigargin, it was investigated whether this compound can be used to suppress production of secreted IL-12 dimer forms.

Celecoxib was dissolved in DMSO and added to recombinant HEK293 cells over a concentration range from 10 μM to 100 μM. As a control DMSO-only treated cells were used. Celecoxib concentrations were chosen on the basis of available literature data, and coincide with optimal activity of the compound in various cell-based systems. Two hours later cells were induced with Ponasterone A to produce IL-12 α/β or β/β dimer forms. After 16 hrs of additional incubation, culture medium was collected and assessed for the presence of IL-12 dimer forms by means of non-reducing SDS-PAGE and immunoblot. This showed that Celecoxib suppressed production of

secreted IL-12 β/β homodimers by >95% when used at a concentration equal to or larger than 30 μM; and of secreted IL-12 α/β heterodimers by >95% when used at a concentration equal to or larger than 10 μM. Secretion of IL-12 α and β monomer forms was not suppressed by Celecoxib. Toxicity as measured with the MTT assay was visible when cells were treated for 16 hrs with a concentration of Celecoxib equal to or larger than 100 μM.

The mechanistic basis of this effect was investigated by analysing intracellular dimer formation in cells treated with Celecoxib. Cells were treated with Celecoxib and induced with Ponasterone A as described above. After 16 hrs, cells were lysed with Triton-X-100-containing buffer, and lysates were submitted to non-reducing SDS-PAGE and immunoblotted. IL-12 dimer forms were present in the cell lysates of both untreated cells and in cells treated with Celecoxib, and no difference was observed in the respective amounts. All data taken together, this suggests that Celecoxib blocks secretion of IL-12 dimer forms by a mechanism that involves intracellular retention of preformed IL-12 dimer forms, and not by inhibition of dimer formation.

It was then investigated whether $Ca^{2+}$-dependent chaperones are possibly involved in the effects conferred by Celecoxib. In view of the results detailed above, it was hypothesized that the disturbance of the $Ca^{2+}$-balance in the ER by Celecoxib (i) is unlikely to block interaction of IL-12 with chaperones during dimer formation (as

formation of the intracellular dimer is uncompromised), but (ii) is likely to prevent the release of $Ca^{2+}$-dependent ER chaperones from IL-12 dimer forms. The resulting complex of IL-12/chaperones is then possibly retained in the ER through interaction with a receptor in the ER that specifically interacts with the KDEL sequence present in most chaperones. To investigate this hypothesis, cells were treated with Celecoxib for 2 hours, induced with Ponasterone A, and incubated for a further 16 hours. Cells were lysed, and intracellular IL-12 was immunoprecipitated from the lysates by means of $Ni^{2+}$-NTA-agarose that binds the C-terminal hexahistidine sequence. Purified immunoprecipitates were submitted to reducing SDS-PAGE and blotted onto PVDF membranes. These membranes were probed for the presence of various ER chaperones by incubating with antibodies specific for calreticulin, Grp78/BiP and Grp94. This showed that the amount of calreticulin – but not of Grp78 and Grp94 – associated with IL-12 dimer forms was significantly increased in cells treated with Celecoxib compared to untreated cells. The amount of calreticulin co-immunoprecipitated with IL-12 from Celecoxib-treated cells was at least 5 times larger than that in control cells.

The present data demonstrates that Celecoxib efficiently suppresses secretion of IL-12 $\alpha/\beta$ and $\beta/\beta$ dimer forms by a post-transcriptional and post-translational mechanism that involves $Ca^{2+}$-dependent intracellular retention of IL-12 dimers. Maximal IL-12-suppressive effects are observed at a

physiological Celecoxib concentration in the absence of any obvious toxic effects.

For oral administration, the medicament according to the invention may be in the form of, for example, a tablet, capsule suspension or liquid. The medicament is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are capsules, tablets, powders, granules or a suspension, with conventional additives such as lactose, mannitol, corn starch or potatoes starch; with binders such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators such as corn starch, potaote starch or sodium carboxymethyl-cellulose; and with lubricants such as talc or magnesium stearate. The active ingredient may also be administered by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable carrier.

For intravenous, intramuscular, subcutaneous, or intraperitioneal administration, the compound may be combined with a sterile aqueous solution which is preferably isotonic with the blood of the recipient. Such formulations may be prepared by dissolving solid active ingredient in water containing physiologically compatible substances such as sodium chloride, glycine, and the like, and having a buffered pH compatible with physiological conditions to produce an aqueous solution, and rendering said

solution sterile. The formulations may be present in unit or multi-dose containers such as seated ampoules or vials.

If the inflammatory disease is localized in the G.I. tract, the compound may be formulated with acid-stable, base-liable coatings known in the art which began to dissolve in the high pH intestine. Formulations to enhance local pharmacologic effects and reduce systemic uptake are preferred.

Formulations suitable for administration conveniently comprise a sterile aqueous preparation of the active compound which is preferably made isotonic. Preparations for injections may also be formulated by suspending or emulsifying the compounds in non-aqueous solvent, such as vegetable oil, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol.

Formulations for topical use include known gels, creams, oils, and the like. For aerosol delivery, the compounds may be formulated with known aerosol exipients, such as saline and administered using commercially available nebulizers. Formulation in a fatty acid source may be used to enhance biocompatibility. Aerosol delivery is the preferred method of delivery for epithelial airway inflammation.

For rectal administration, the active ingredient may be formulated into suppositories using bases which

are solid at room temperature and melt and dissolve at body temperature. Commonly used bases include cocoa butter, glycerinated gelatin, hydrogenated vegetable oil, polyethylene glycols of various molecular weights, and fatty esters of polyethylene stearate.

The dosage form and amount can be readily established by reference to known inflammatory disease treatment or prophylactic regiments. The amount of therapeutically active compound that is administered and the dosage regimen for treating a disease condition with the compounds and /or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, the location of the inflammatory disease, as well as the pharmacokinetic properties of the individual treated, and thus may vary widely. The dosage will generally be lower if the compounds are administered locally rather than systemically, and for prevention rather than for treatment. Such treatments may be administered as often as necessary and for the period of time judged necessary by the treating physician. One of skill in the art will appreciate that the dosage regime or therapeutically effective amount of the inhibitor to be administrated may need to be optimized for each individual. The pharmaceutical compositions may contain active ingredient in the range of about 0.1

to 2000mg, preferably in the range of about 0.5 to 500mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100mg/kg body weight, preferably between about 0.1 and about 50mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

The invention is not limited to the embodiments hereinbefore described which may be varied in detail without departing from the invention.

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